ORIGINAL ARTICLES

Expressions of G2A and OGR1 in peripheral blood cells of patients with hypoxia-induced pulmonary hypertension

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Abstract

Objective: To detect the expression changes of proton-sensing receptor G protein-coupled receptor 2A (G2A) and ovarian cancer G protein-coupled receptors 1 (OGR1) in human peripheral blood cells of patients with hypoxia-induced pulmonary hypertension (HPH).

Methods: Thirty-one patients with HPH were enrolled for IPH group, 16 males and 15 females, aged (65.19 ± 5.86) years; and 30 healthy people were enrolled for control group (NC group), 15 males and 15 females, aged (63.47 ± 6.16) years. The peripheral blood samples were collected and the mRNA expressions of G2A and OGR1 were determined by using real-time fluorescent quantitative PCR. The pulmonary arterial pressure (PAP) of HPH group was detected with echocardiography for the analysis of blood gas and pulmonary function testing. Human peripheral blood was collected to detect the mRNA levels of G2A, OGR1 and the serum levels of tumor necrosis factor-α (TNF-α).

Results: $PaCO_2$ was increased significantly in HPH group than that in NC group ($p < .05$). The percentage of forced expiratory volume in 1 s in predicted value (FEV1 pro%) and the ratio of FEV1/forced vital capacity (FVC) in HPH group were significant lower than those in NC group ($p < .05$). The expressions of peripheral blood G2A mRNA and TNF-α in HPH group were increased dramatically than those in NC group ($p < .05$). The expressions of OGR1 mRNA in peripheral blood had no difference between HPH group and NC group. The expressions of G2A mRNA and TNF-α in HPH group were positively related to pulmonary artery pressure significantly.

Conclusions: The expression of proton-sensing receptor G2A and the level of TNF-α were increased in peripheral blood cells of patients with pulmonary hypertension. The expressions of TNF-α and G2A had positive correlations with pulmonary artery pressure.

Key Words: Hypoxia-induced pulmonary hypertension, Proton-sensing receptor, G2A, OGR1, TNF-α

Hypoxic-induced pulmonary hypertension (HPH) is the imbalance of various vasoconstrictor factors resulting from hypoxia-induced injury of vascular endothelial cells and synthesis and secretion of vascular endothelium, leading to increased pulmonary arterial pressure (PAP) and resistance. It is the central link of the pathogenesis of pulmonary heart disease, which is a serious threat to human health.[1–3] The morbidity and mortality of HPH caused by chronic obstructive pulmonary disease (COPD) are high, which can bring a heavy burden to families and society. So far, there is no effective treatment. Therefore, to clarify the mechanism of HPH and find an effective therapy method for the prevention and treatment of HPH is essential.

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The ovarian cancer G protein-coupled receptor 1 (OGR1) subfamily is a newly discovered class of proton-sensitive G protein-coupled receptors that include OGR1, T cell death associated gene 8 (TDAG8), GPR4 and protein-coupled G2A (G2 accumulation) which induces cell arrest in G2/M phase, which are collectively defined as OGR1 subfamily receptors.[4,5] They are widely expressed in vascular endothelial cells and smooth muscle cells, and are closely related to the physiological and pathological processes such as tumorigenesis and metastasis, cytoskeletal reorganization, immune system and vascular system functions, and are important for the regulation of biological functions. The subfamily performs different functions through the induction of multiple intracellular signal transduction pathways by several heterotrimeric G proteins, including Gs, Gi, Gq and G12/13. At present, there are few studies on the expression of OGR1 subfamily in the vascular system. In this study, the expression of G2A and OGR1 in peripheral blood cells of HPH patients was detected to understand its changes in HPH, so as to provide theoretical evidence for further diagnosis and treatment of HPH.

1 Objects and methods

1.1 Research objects

A total of 31 cases of HPH patients with COPD in the Department of Respiratory and Critical Care Medicine of Baogang Hospital, from November 2013 to November 2014, were enrolled in the study, including 16 males and 15 females, with an age of (65.19 ± 5.86) years. They also met the following inclusion criteria: diagnostic criteria for COPD group of the Chinese Medical Association for respiratory diseases,[6] echocardiography predicted pulmonary artery systolic pressure (PASP) $\geq$ 40 mmHg, arterial blood gas analysis consistent with the diagnostic criteria of respiratory failure.[7] Exclusion criteria: bronchial asthma, bronchiectasis, congestive heart failure, tuberculosis, bronchiolitis obliterans, diffuse bronchiolitis, pulmonary hypertension due to left heart disease, chronic thromboembolic pulmonary hypertension, connective tissue-caused pulmonary hypertension, liver, kidney and other chronic diseases, combined with a serious systemic disease. During the same period, 30 healthy subjects in our hospital were enrolled in control group (NC group), including 15 males and 15 females, with an age of (63.47 ± 6.16) years. This study was approved by Inner Mongolia Baogang Hospital Medical Ethics Committee, all subjects signed a written informed consent.

1.2 Research methods

1.2.1 Observation of indicators

(1) Echocardiography examination: All patients were examined by echocardiography on the first day of admission, including right heart function index, systolic right atrial pressure, maximum pressure difference of three cusp regurgitation and PASP. PAP was assessed by echocardiography using tricuspid regurgitation to assess PASP without right ventricular outflow tract obstruction and pulmonary stenosis. The formula for right ventricular systolic pressure is: RVSP = ΔP + SRAP (RVSP = right ventricular systolic pressure; SRAP = systolic right atrial pressure; ΔP = maximum tricuspid regurgitation pressure difference). SRAP was 0.667 kPa (5 mmHg) for mild tricuspid regurgitation, SRS was 1.333 kPa (10 mmHg) for moderate tricuspid regurgitation, and SRAP was 2.000 kPa for severe tricuspid regurgitation (15 mmHg). (2) Blood gas analysis: The arterial blood was extracted immediately after entering the group, and the arterial blood gas analysis was performed by the German Roche cobasbl23 blood gas analyzer. (3) Examination of pulmonary function: The Jaeger lung function meter manufactured by Germany's Yager Company was used. All patients were tested for pulmonary function on the first day of hospitalization and the forced expiratory volume in one second as the predicted percentage (FEV1 pro%), forced vital capacity as the predicted percentage (FVC pro%), and FEV1/FVC were observed.

1.2.2 Detection of mRNA expression of G2A and OGR1 in peripheral blood by real-time PCR

(1) 4 ml of peripheral blood anticoagulant was collected for all the subjects at the time of being arranged to the group. RNA stabilizer was added to 2 ml anticoagulant and placed at room temperature for 2 h, and then was stored at -70°C. The other 2 ml anticoagulant blood was centrifuged at 1,000 r/min × 15 min. After the blood was collected, the supernatant was subdivided into the EP tube and cryopreserved at -70°C. The extraction of the total RNA in the blood was carried out and cDNA was synthesized according to the operation description of the RNA prep pure blood total RNA extraction kit (Tian Gen Biochemical Co., Ltd.). On the basis of NCBI gene bank, it was numbered NM-002046.3 (GAPDH). According to the design requirements of SYBR Green I quantitative PCR primers, Primerblast software was used to design the proton sensing receptor and internal reference gene specific quantitative PCR primer: TH-G2A-F: 5'-TTTGCCATCCCTCTCTCCAT-3', TH-G2A-R: 5'-GCTCTGCTTGATGCTCCTGAA-3', length 236 bp; TH-OGR1-F: 5'-TCGCCAAGGGCGTTTTC-3', TH-OGR1-R: 5'-GTCGGCGACGCAGTTGA-3', length 148 bp; GAPDH-F: 5'-ATGAACATGGCTGTGCCTTTG-3', GAPDH-R: 5'-AGCACCCCTAACCTTGTGC-3', 116 bp. The above primers were synthesized by Shanghai Sangon Bioengineering Co., Ltd.

(2) PCR reaction conditions: pre-deformation: 95°C, 30 s; deformation: 95°C, 5 s; annealing: 60°C, 20 s; extension: 72°C, 30 s, a total of 50 cycles.

(3) The expression level of the target gene GAPDH was analyzed by real-time PCR amplification curve calculation, with 4 parallel wells in each group. The target gene CT value of each sample was subtracted from the GAPDH CT value of the reference gene to calculate the expression level of the target gene, and the result was calculated by ACT method. The average relative content (%) = relative content of unknown sample/relative content of control sample = $2^{-\Delta CT}$ where ΔCT of unknown sample = unknown CT sample - CT internal reference.

1.2.3 The serum levels of TNF-α protein detected with ELISA

The operation was in accordance with TNF-α ELISA quantitative detection kit (Beijing Kanu Biotechnology Co., Ltd.).

1.3 Statistical processing

The data were processed by statistical software SPSS 17.0. The experimental data were expressed as mean ± standard deviation ($\bar{x}$ ± s), and the measurement data were analyzed by two independent samples *t*-test or approximate *t*-test. The correlation analysis was based on linear correlation analysis.

2 Results

2.1 Clinical features comparison of HPH group and NC group

There was no significant difference in age and sex between HPH group and NC group. $PaCO_2$ in HPH group was significantly higher than that in NC group ($p < .05$). FEV1 pro% and FEV1/FVC (%) in HPH group were significantly lower than those in NC group ($p < .05$, see Table 1). PASP in HPH group was (60.03 ± 15.79) mmHg. PASP could not be calculated in healthy subjects in NC group because there was no tricuspid regurgitation in the cardiac ultrasonography.

2.2 Comparison of the expression of peripheral blood G2A and OGR1 mRNA of HPH group and NC group

Quantitative PCR detection of G2A and OGR1 was performed, using GAPDH RNA as an internal control. The expression of G2A in HPH group was significantly higher than that in NC group ($p < .01$). There was no difference in OGR1 expression in peripheral blood between HPH group and NC group (see Table 2 and Figure 1).

2.3 Serum content of TNF-α detected with ELISA

The average content of TNF-α (0.26 ± 0.09) ng/L in HPH group was significantly higher than that in NC group (0.17 ± 0.07) ng/L ($p < .01$).

2.4 Correlation analysis between the expression of proton-sensing receptors, the content of TNF-α and PASP in HPH group

After Pearson linear correlation analysis, there was a significant positive correlation between PASP and G2A together with TNF-α content of peripheral blood in HPH group ($p < .01$, see Figure 2), while there was no correlation between OGR1 and PASP in HPH group.

Table 1: Comparison of clinical characteristics between HPH and NC group ($\bar{x}$ ± s)

Group	n	Gender (male/female)	Age (years)	$PaCO_2$ (mmHg)	FEV1 pro (%)	FEV1/FVC (%)
NC	30	15/15	63.47 ±6.16	39.40 ±3.39	109.0 ±18.3	82.6 ±3.6*
HPH	31	16/15	65.19 ±5.86	54.54 ±20.88*	39.6 ±12.8*	55.5 ±7.7*

Note. HPH: hypoxia induced pulmonary hypertension; NC: normal control; $PaCO_2$: pressure of carbon dioxide; FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity; $p < .05$ vs. NC group

Table 2: mRNA expression quantity between HPH and NC group ($\bar{x}$ ± s)

Group	n	G2A	OGR1
NC	30	7.33 ±5.39	1.40 ±0.98
HPH	31	33.36 ±12.35**	1.17 ±0.94

Note. HPH: hypoxia induced pulmonary hypertension; NC: normal control; G2A: G2 accumulation; OGR1: ovarian cancer G protein coupled receptor 1; $p < .01$ vs. NC group

3 Discussion

COPD is a disease of lung chronic inflammation caused by harmful particles and gases in the atmosphere, leading to pulmonary remodeling. Long-term hypoxia leads to persistent pulmonary vascular resistance and pulmonary hypertension and remodeling of pulmonary vascular structure, which are called HPH during clinic. Previous study showed that

the thickening pulmonary artery wall of SD rats under hypoxic low pressure culture made the lumen seriously narrow in the hypoxia group, while in the normal group, the pulmonary artery wall was normal.[8] A large number of studies have confirmed the existence of airway inflammation in COPD patients. The relationship between inflammatory reaction and pulmonary hypertension with COPD is receiving more and more attention. In order to adapt and resist with hypoxia, the body will start the inflammation and immune response. It has been found that a large number of proinflammatory cytokines, such as TNF-α, IL-1 and IL-6, are significantly increased in HPH patients and rat models of sputum and bronchoalveolar lavage fluid. These factors can further enhance the inflammatory response.[9] TNF-α, a cytokine produced by alveolar macrophages, is a pro-inflammatory cytokine. In this study, the expression of TNF-α in HPH group was significantly higher than that in NC group, indicating that TNF-α was involved in the pulmonary vasoconstrictor response and the formation of pulmonary hypertension under chronic hypoxia.

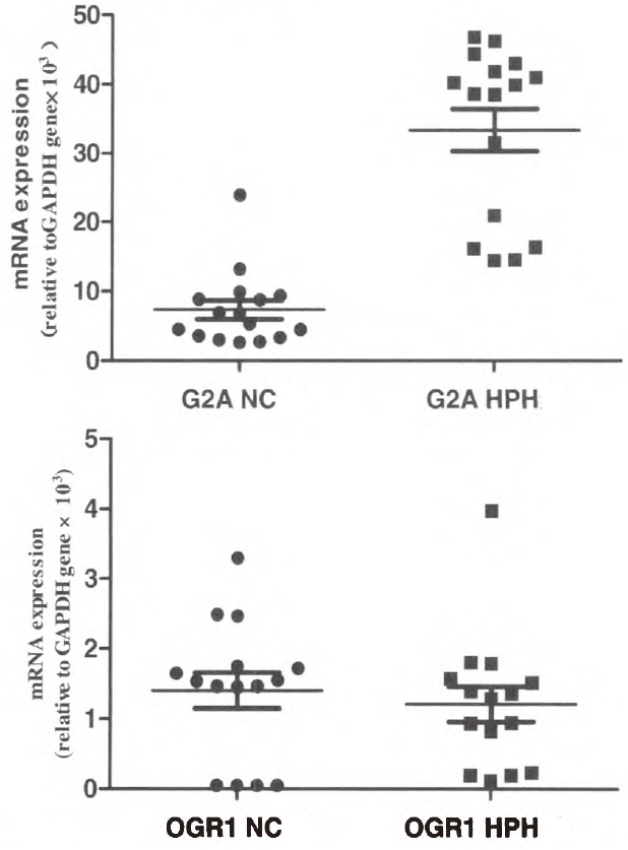

Figure 1: Comparison of mRNA expression quantity between HPH and NC group ($\bar{x} \pm s$)
HPH: hypoxia induced pulmonary hypertension; NC: normal control; G2A: G 2 accumulation; OGR1: ovarian cancer G protein-coupled receptor 1

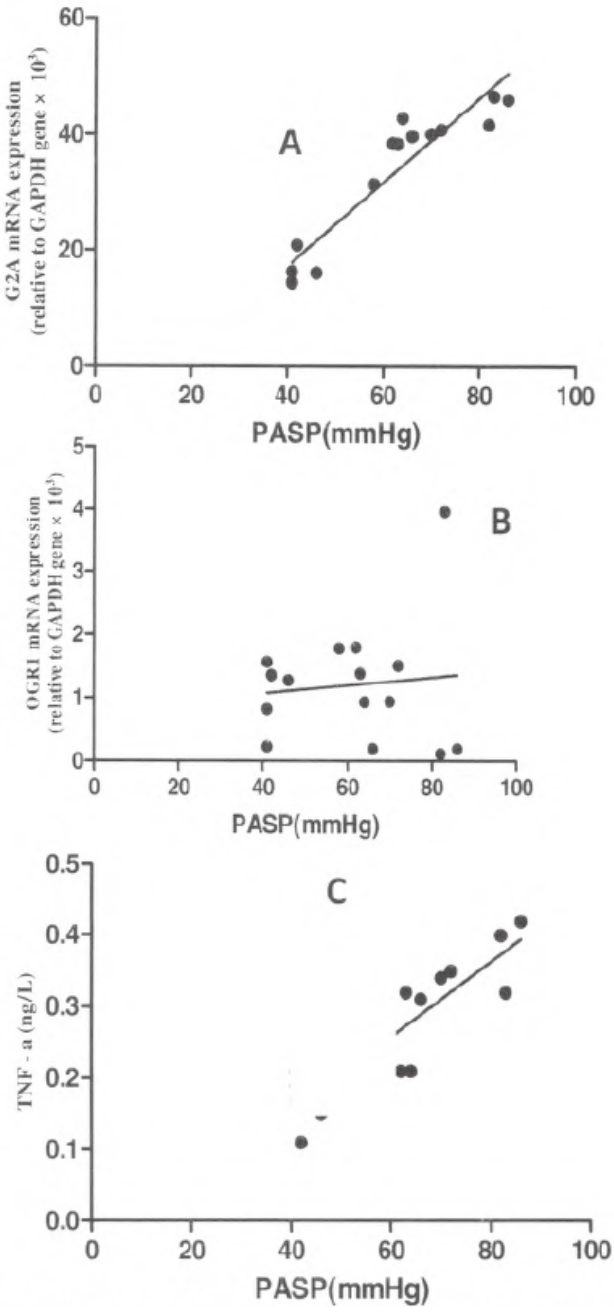

Figure 2: Correlation of expression level of the proton-sensing receptor, TNF-α and pulmonary artery pressure in HPH group
A: G2A and PASP; B: OGR1 and PASP; C: TNF-α and PASP; TNF-α: tumor necrosis factor-α; HPH: hypoxia induced pulmonary hypertension; PASP: pulmonary artery systolic pressure

This study confirmed that G2A was highly expressed in HPH group, and G2A expression was positively correlated with pulmonary artery pressure, suggesting that G2A was involved in the development of HPH. The expression of G2A in patients with HPH was significantly higher than that in NC group, and it was considered that patients with pul-

monary hypertension often accompanied by respiratory failure. Anoxia or anoxia associated with carbon dioxide retention caused by respiratory failure, results in acidic environment within the body. Under hypoxia and inflammation, a large amount of lactic acid is produced by anaerobic glycolysis due to hypoxia in the cell microenvironment, leading to the formation of an acidic microenvironment at the site of inflammation.[10] The acidic microenvironment can extensively modulate the proinflammatory and anti-inflammatory properties of various cell types, thereby exacerbating or improving inflammation and its associated disease status.[11,12] Immune inflammatory response is a complex network, we can achieve the best therapeutic effect by looking for and inhibiting the activation of one or more of the key signaling pathways that G2A participates in. The detection of G2A mRNA expression can be early used to discover the symptoms of patients with pulmonary hypertension. The relationship between proton-sensing receptors, inflammatory factors and HPH can provide a basis for clinical prevention and treatment of COPD complicated with pulmonary hypertension. At the same time, this study provides the basis for further study on the role of proton-sensing receptors in the occurrence and development of pulmonary hypertension, and provides a theoretical basis for early intervention of pulmonary hypertension in molecular level.

Conflicts of Interest Disclosure

The authors have no conflicts of interest related to this article.

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